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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/796,164	02/06/97	STAMLER J	[illegible]

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EXAMINER	
CELSA, D	

ART UNIT	PAPER NUMBER
1654	28

DATE MAILED: 10/29/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

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Office Action Summary

Application No.	Applicant(s)
08/796,164	Stamler et al.
Examiner	**Group Art Unit**
Bennett Celsa	1654

☒ Responsive to communication(s) filed on *Aug 30, 1999*.

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *10-22, 24-29, 40-46, and 63-68* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *10-22, 24-29, 40-46, and 63-68* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

Response to Amendment

Applicant's after final amendment dated 8/30/99 in paper no. 25 has been entered.

Applicant's Petition (dated 4/26/99 in paper no. 21) of the Examiner's Supplemental Restriction of claims 42, 45 and 46 presented in the Final Rejection dated 3/30/99 in paper no. 20 has been granted.

Withdrawal of Final Rejection

In order to best address issues raised by claims 42, 45 and 46 and those issues discussed during the in personal interview conducted with the Examiner on August 5, 1999 with Dr. Stamler, Carol Egner and David Brook, the final status of the paper no. 20 office action is hereby withdrawn.

Status Of The Claims

Claims 10-22, 24-29, 40-46 and 63-68 are currently pending and under consideration.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 63-66 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention (e.g. NEW MATTER REJECTION)

The specification disclosure (bottom of page 72-top of 73) of a specific multistep protocol for forming SNO-Hemoglobin does not support the generic method claim 63.

The specification disclosure (bottom of page 73-top of 74) and especially page 73, lines 14-20 which disclose a specific multistep protocol for making nitrosylhemoglobin, does not support the generic method of claim 64, nor does the specification support the "heme:NO ratio of greater than about 14" as presently claimed.

The specification disclosure (bottom of page 75- top of page 76) which discloses a specific multistep protocol for making SNO-Hb which includes specific buffer and amounts does not support the generic method of new claim 65. Similarly, specific support for 18.3uM hemoglobin fails to support the range of "greater than about 18uM" in dependent claim 66.

The specification disclosure in Example 16 (page 73) discloses a specific multistep method of making predominantly nitrosylhemoglobin which requires specific: hemoglobin A amounts, buffer and amounts thereof; pH (e.g. 7.4); timing etc. . Accordingly, the generic method of new claim 67 is not supported.

Additionally, the claimed heme-ratio of "at least about 70:1" in BOTH claims 67 and 68 is not supported by the disclosed "approximately 7:1" since these terms appear to possess different scope e.g. the claimed invention scope is broader.

The claimed scope of nitrosylhemoglobin compositions of new claim 68 is not supported by a single example (e.g. Example 16) drawn to a specific buffer (e.g. phosphate) which results in a mixture of ntirosylhemoglobin and some oxidized hemoglobin. Applicant's claim which includes both reactants (e.g. heme:NO) and final product (e.g. nitrosylhemoglobin) is not supported by the specification.

A response to this rejection must include cancellation of the newly added subject matter.

3. Claims 10-15, 42, 45, 46 and 63-68 are rejected under 35 U.S.C. 112, first paragraph, as based on a disclosure which is not enabling.

Critical or essential method parameters: which include reagent concentration, pH and presence and concentration of buffer, necessary to the practice of the invention, but not included in the claim(s) is not enabled by the disclosure. See *In re Mayhew*, 527 F.2d 1229, 188 USPQ 356 (CCPA 1976).

For instance the specification specifically states on page 77, lines 25-30 that "... the balance between oxidation and nitrosothiol formation is dependent upon the ratio of nitric oxide to hemoglobin and the buffer environment. Accordingly, **both** buffer presence in effective

amounts and NO: heme concentrations are critical to practicing the presently claimed methods and the achieving of the presently claimed compositions.

Similarly, specification pages 73-74 disclose that NO:heme ratios and whether the method is performed under anaerobic or aerobic conditions are critical since different ratios and atmospheric conditions result in different final products (e.g. in site and degree of nitrosylation and degree of oxidation).

Finally, the submitted Stamler Declaration provides further evidence that the choice of nitrosating agent, the amount of agent vis a vis hemoglobin concentration, and pH are critical toward obtaining stable S-nitrosylation of hemoglobin.

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 45 and 68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A. In claims 45 and 68 "heme:NO" and "NO:heme" ratios lack antecedent basis regarding the components that constitute the ratio.

Claim Rejections - 35 USC § 102

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371© of this title before the invention thereof by the applicant for patent.

6. Claims 10, 11, 13, 14, 43, 44, 63, 65 and 66 are rejected under 35 U.S.C. 102(b) as being anticipated by or alternatively under 35 USC 103 as being obvious over Chem. Res Tox. 1990 Vol. 3, pages 289-291.

The reference discloses a method of transferring the nitrosyl group to sulfur (as well as oxygen, nitrogen and sulfur) of heme proteins, including hemoglobin to thus form SNO-hemoglobin by reacting hemoglobin in pH 7.4, 0.01 buffer (see table 1) under anaerobic conditions in excess nitric oxide (e.g. @ 2x10-3M) (e.g. see page 289 under "Results and Discussion"). The reference NO and hemoglobin concentrations are within the scope of the presently claimed invention. It is noted that a "composition" comprising an S-nitrosylated hemoglobin in which additionally other moieties (e.g. carbon, oxygen and nitrogen) are within the scope of the present composition claims. The isolation of the nitrosated hemoglobin species and/or the spectrophotometric determination (e.g. page 290) presumably in air would be expected to form the the oxyhemoglobin species. Alternatively, it would have been obvious to one of ordinary skill in the art to generate the oxygenated hemoglobin species by air oxidation especially

since the reference specifically points to a nitrosation process which occurs under aerobic conditions.E.g see page 290, left column and footnote 4. The degree of heme oxidation (e.g. "nondetectable") of the reference nitrosylated hemoglobin would be met inherently by the reference which utilizes a method within the scope of the presently claimed invention. The Examiner lacks the facilities to do testing.

7. Claims 42 and 45 are rejected under 35 U.S.C. 102(b) as being anticipated by Moore et al., J.Biol. Chem. Vol. 251, No. 9, (5/76) pages 2788-2794..

Moore et al. disclose a "stock nitrosylhemoglobin" solution which comprises nitrosyl-deoxyhemoglobin. Thus, the reference composition is within the scope of the presently claimed invention. Claims 42 and 45 are product by process claims, wherein a particular NO-heme concentration is recited to be used to generate the final product. However, product-by-process claims are viewed by the PTO as product claims.

8. Claims 42 and 45 are rejected under 35 U.S.C. 102(b) as being anticipated by Sharma et al., J. Biol. Chem. Vol. 253, No. 18 (9/78) pages 6467-72.

Sharma et al. discloses buffered HbNO solutions (e.g. see page 6468, left column) which can be made by NO addition to deoxygenated hemoglobin or by titration of deoxyhemoglobin with NO gas. Regardless of the reference method of manufacture, the reference HbNO composition comprises buffered nitrosyl-deoxyhemoglobin. Thus, this composition is within the scope of the presently claimed invention.

Claims 42 and 45 are product by process claims, wherein a particular NO-heme concentration is recited to be used to generate the final product. However, product-by-process claims are viewed by the PTO as product claims.

9. Claims 16, 20-22, 27-28 and 40 are rejected under 35 U.S.C. 103(a) as obvious over Stamler et al, WO 93/09806 (5/93).

Stamler et al. teach that nitrosylated low molecular weight thiols (e.g. N-acetyl cysteine) serve as NO donating compounds (e.g. delivery of NO) which are therapeutically useful as smooth muscle relaxants, vasodilators and platelet inhibition (e.g. see abstract and pages 1-2 of Stamler).

Similarly to low molecular weight thiols, the Stamler reference further teaches that proteins (including hemoglobin), which are nitrosylated on oxygen, carbon or nitrogen sites possess the same therapeutic utility as nitrosylated/nitrated low molecular weight thiol compounds. (E.g. see page 6, lines 13-15; page 7, lines 17-21; and claims).

The reference specifically discloses the use of nitrosylated proteins and low molecular weight nitrosating agents (e.g. see pages 1-2; page 24, lines 10-16) preparations thereof for the treatment of disorders by increasing oxygen capacity and transport; modulating CO and NO to tissues; scavenging radicals and vasodilation such as treating lung diseases (e.g. ARDS) and hypoxic disorders (E.g. see pages 19-25 and claims).

Further it is known in the art that hemoglobin is involved in regulating oxygen metabolism by its ability to bind reversibly to blood oxygen and thus facilitate the capability of blood to transport oxygen to bodily tissues (e.g. see bottom of page 19-top of page 20).

Accordingly, it would have been obvious to combine a low molecular weight thiol or nitrosothiol with either hemoglobin or nitrosated hemoglobin to deliver oxygen or NO (e.g. claim 16) since the Stamler reference teaches the use of the same compounds separately to effectuate the same function.

Additionally, the use of Nitrosated/Nitrated proteins, including nitrosated/nitrated hemoglobin to deliver NO to tissues (e.g. claim 40) in order to effectuate the treatment of abnormalities or diseases which are mediated by nitric oxide and oxygen metabolism (e.g. lung disease, sickle cell anemia, heart disease, high blood pressure etc.) would have been obvious since the reference discloses the use of nitrosated proteins, including nitrosated hemoglobin, to treat such disease states.

10. Claim 41 is rejected under 35 U.S.C. 103(a) as being unpatentable over Stamler WO93 as applied to claims 16, 20-22, 27-28 and 40 above, and further in view of Moore et al., J.Biol. Chem. Vol. 251, No. 9, (5/76) pages 2788-2794 or Sharma et al., J. Biol. Chem. Vol. 253, No. 18 (9/78) pages 6467-72.

The Stamler reference disclosure discussed in the above obviousness rejection over Stamler alone is hereby incorporated by reference in its entirety.

The Stamler reference although disclosing the use of nitrosyl-heme containing NO donors to deliver NO or its biological equivalent to tissues (e.g. present claim 40) fails to specifically disclose the use of nitrosylhemoglobin (e..g dependent claim 41)..

However, nitrosylhemoglobin compositions are conventionally known in the art. E.g. See the Moore and Sharma references.

One of ordinary skill in the art would be motivated to selected nitrosylhemoglobin to deliver NO to tissues in view of the Stamler reference which suggests that this compound would be expected to function as an NO-donating compound.

Accordingly, it would be obvious for one of ordinary skill in the art at the time of applicant's invention to select available nitrosylhemoglobin compositions to deliver NO as taught by Stamler.

11. Claims 10-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stamler (WO 93).

The presently claimed invention is directed to producing a composition comprising either SNO-Hb[FeII]O2 (produced in the presence of oxygen) or SNO-Hb[FeII] (produced in the absence of oxygen) by reacting "excess nitrosating agent" with purified hemoglobin (e.g. claims 10-11 and 13-14). Claims 12 and 15 specifically select a low molecular weight S-nitrosothiol as the nitrosating agent.

Stamler discloses different methods for thiol nitrosylation of proteins (as disclosed on page 30-31) which include:

1. reaction of nitrosylating agent (e.g. equimolar amounts of acidic NaNo2 as nitrosating agent in a buffered saline at pH 7.4 for tPA);

2. exposure of the protein (e.g. tPA to NO gas in buffered saline)

With regard to the above, Stamler further notes that other oxides of nitrogen can be utilized (e.g. NOCL, N2O3) as well as other nitroso equivalents.

However, the above two reference methods for thiol nitrosylation fail to disclose the use of "excess" nitrosating agent, and preferably the selection of a low molecular weight S-nitrosothiol as the nitrosating agent for thionitrosylation of hemoglobin.

But the Stamler reference (e.g. Example 19 on pages 58-59) specifically discloses the preferential selection of a low molecular weight S-nitrosothiol (e.g. SNOAC) instead of acidic Na NO2 as utilized for tPA due to reduced ability of the SNOAC as compared with acidic nitrate to bind at the redox metal which reduces oxygen binding affinity.

Further, the use of "excess nitrosating agent" in either reaction 1. or 2 above is suggested by the Stamler reference since providing a greater concentration of NO serves to enhance the therapeutic efficacy of the nitrosylated proteins (e.g. see bottom of page 23-top of 24)

It is further noted that the use of higher pH values (e.g. pH 7.4) than that utilized in the thionitrosylated hemoglobin example (e.g. pH 6.9 Example 19) is also suggested by the reference since thionitrosylated proteins are known to be stable under physiological conditions (e.g. TBS,

pH 7.4, room temperature: see page 31) and further the reference discloses the use of pH7.4 in the steps analogous to that of Example 19: see page 30, lines 20-27; page 33, lines 20-26).

Optimization of reaction conditions is within the skill of the art.

Additionally, it is a matter of obvious design choice to select anaerobic conditions for making a deoxygenated hemoglobin derivative and aerobic conditions when desiring to make an oxygenated hemoglobin derivative.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to synthesize thionitrosylated hemoglobin by using "excess" nitrosating agent, and preferably a low molecular weight S-nitrosothiol, and to further optimize pH during nitrosylation to utilize physiological conditions to form a more stable nitrosylated oxy/deoxy hemoglobin.

12. Claims 17-19 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Feola et al. , U. S. Pat. No. 5,439,882 (8/95: filed 5/93 or earlier) and Stamler, and if necessary further in view of Moore or Sharma.

Feola et al. disclose the state of the prior art regarding "blood substitutes" as being an emergency resuscitative fluid that:

a. Restores blood volume;

b. Transports oxygen;

c. Reduces vasoconstiction. See Feola col. 1.

Feola et al. disclose the use of "blood substitutes" which comprises hemoglobin alone or combined with glutathione as a blood substitute to treat blood disorders (e.g. sickle cell anemia) (e.g. see Abstract, examples and columns 1 and 7).

The Feola reference "blood substitute" composition and intended use thereof (e.g. treat sickle cell anemia) differs from the presently claimed invention which utilizes nitrosated hemoglobin alone or with a low molecular weight S-nitrosothiol instead of hemoglobin or hemoglobin combined with glutathione.

However, Stamler et al. teach that nitrosylated low molecular weight thiols (e.g. N-acetyl cysteine) serve as NO donating compounds (e.g. delivery of NO) which are therapeutically useful as smooth muscle relaxants, **vasodilators** and platelet inhibition (e.g. see abstract and pages 1-2 of Stamler).

The Stamler reference specifically discloses the use of nitrosylated proteins and low molecular weight nitrosating agents (e.g. see pages 1-2; page 24, lines 10-16) preparations thereof for the treatment of disorders by increasing oxygen capacity and transport; modulating CO and NO to tissues; scavenging radicals and vasodilation such as treating lung diseases (e.g. ARDS) and hypoxic disorders (E.g. see pages 19-25 and claims).

Thus, the Stamler et al. reference provides the skilled artisan with motivation to utilize nitrosated hemoglobin alone or with a low molecular weight S-nitrosothiol to make a blood substitute for treating sickle cell anemia in order to increase blood volume, oxygen delivery and

reduce vasoconstriction as effected by nitrosated hemoglobins alone or in conjunction with a nitrosothiol.

Further, nitrosylated hemoglobin preparations, e.g. nitrosylhemoglobin compositions, are conventionally known in the art. E.g. See the Moore and Sharma references.

Accordingly, it would have been obvious to the skilled artisan at the time of applicant's invention to make a blood substitute comprising nitrosated hemoglobin alone or in conjunction with a low molecular weight nitrosothiol for their expected benefits as suggested by the Stamler reference and in analogous manner as the Feola reference composition..

13. Claims 24-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Feola et al. and Stamler, and if necessary further in view of Moore or Sharma.as applied to claims 17-19 and 26 above, and further in view of Chem. Res Tox. 1990 Vol. 3, pages 289-291.

As discussed above, and as hereby incorporated by reference in its entirety, the Stamler reference suggest the use of nitrosylated hemoglobin alone or combined with a thio containing compound in order to function equivalently to the Feola hemoglobin preparation as a blood substitute useful to treat sickle cell anemia.

Claims 24-26 are drawn to the use of a thionitrosylated hemoglobin as the nitrosating agent to be employed in the blood substitute.

However, S-nitrosylation of hemoglobin serves to increase hemoglobin-oxygen binding as taught by Stamler et al. (E.g. see pages 19-20).

Additionally, Stamler discloses different methods for thiol nitrosylation of proteins (as disclosed on page 30-31) which include:

1. reaction of nitrosylating agent (e.g. equimolar amounts of acidic NaNo2 as nitrosating agent in a buffered saline at pH 7.4 for tPA);

2. exposure of the protein (e.g. tPA to NO gas in buffered saline)

With regard to the above Stamler further notes that other oxides of nitrogen can be utilized (e.g. NOCL, N2O3) as well as other nitroso equivalents.

Specifically with regard to hemoglobin, Stamler further discloses (e.g. Example 19 on pages 58-59) the preferential selection of a low molecular weight S-nitrosothiol (e.g. SNOAC) instead of acidic Na NO2 and provides motivation to utilize "excess nitrosating agent" in either reaction 1. or 2 above in order to enhance the therapeutic efficacy of the nitrosylated proteins (e.g. see bottom of page 23-top of 24). Optimization by using higher pH values (e.g. pH 7.4) than that utilized in the specific thionitrosylated hemoglobin example (e.g. pH 6.9 Example 19) is also suggested by Stamler since thionitrosylated proteins are known to be stable under physiological conditions (e.g. TBS, pH 7.4, room temperature: see page 31). See also other Examples which utilize physiological conditions in analogous steps.E.g. page 30, lines 20-27; page 33, lines 20-26).

Further, the Chem. Res Tox. 1990 Vol. 3, pages 289-291 discloses a method of transferring the nitrosyl group to sulfur (as well as oxygen, nitrogen and sulfur) of heme proteins,

including hemoglobin to thus form SNO-hemoglobin; and thus form thionitrosylated hemoglobin compositions. .

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to use thionitrosylated hemoglobin as the nitrosating agent to be employed in a blood substitute in view of the benefits flowing therefrom e.g. enhanced oxygen binding as disclosed by the Stamler reference.

General information regarding further correspondence

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Celsa whose telephone number is (703) 305-7556.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Woodward, can be reached at (703)308-4028.

Any inquiry of a general nature, or relating to the status of this application, should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Bennett Celsa

October 27, 1999

BENNETT CELSA
PRIMARY EXAMINER